IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Hauptmann *et al.*

Appl. No.: 08/249,671

Filed: May 26, 1994

For: **Process for Preparing and Purifying Alpha-Interferon**

Art Unit: 1812

Examiner: Fitzgerald, D.

Atty. Docket: 0652.1350000/RWE/LLK

considered
7/10/97

Second Declaration Under 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, DC 20231

Attn.: BOX AF

Sir:

The undersigned, Rudolf Hauptmann, declares and states that:

1. I am a coinventor of the above-captioned patent application. A copy of my curriculum vitae is attached to the first Declaration under 37 C.F.R. § 1.132 (unsigned) filed November 18, 1996. A signed copy of said first Declaration is filed herewith.

2. I have read and I am familiar with the prosecution of this application, including the Office Action of December 31, 1996, wherein the Examiner rejected claims 1, 3, 17, 19, 25, and 30 as obvious over Miyake *et al.* in view of Chang *et al.*, and further in view of Vandlen *et al.*, Capon *et al.*, and Baxter *et al.*

3. Miyake *et al.* disclose the recovery of IFN expressed from a vector construct comprising IFN-α cDNA ligated to a sequence encoding the alkaline phosphatase (AP) signal

sequence (AP/IFN-α) under the control of an AP promoter (*i.e.*, an AP/AP/IFN-α construct). According to Miyake *et al.*:

> IFN activity of 1.6×10^4 units/liter culture was recovered from the lysate of *E. coli* K12 C600 (pTA524) before the osmotic shock procedure and 7.6×10^3 units/liter culture from the cold water wash. On the other hand, when *E. coli* K12 C600 (pTA1524) was cultivated, IFN activity of 3.05×10^4 units/liter culture was measured from the lysate and 8.0×10^3 units/liter culture from the cold water wash.

See Miyake *et al.* at page 1436, column 1, lines 4-12. It is assumed that Miyake *et al.* used 50 OD's of cells for each measurement.

4. The IFN activity was measured by Miyake *et al.* with the CPE inhibition assay using NIH fibroblast interferon standards. *See* Miyake *et al.*, at page 1431, the paragraph bridging columns 1 and 2. Interferon units are defined internationally. Standards have been established by the National Institute for Biological Standards and Control (NIBSC) and the NIH. Thus, the units described by Miyake *et al.* are comparable to the units obtained as described below.

5. IFN-α that is periplasmically expressed from the AP/STII/IFN-α construct recited in the claims has a biological activity (as measured by the CPE reduction assay) that is identical to the specific activity found for natural human IFN-α (2.3×10^9 units/mg). As described in said first Declaration, when IFN-α was expressed from *E. coli* HB101 containing the AP/STII/IFN-α construct, 5 to 10 mg/50 OD/liter of IFN-α was obtained. Thus, the yield of IFN-α obtained from this construct (5 to 10 mg/50 OD/liter) corresponds to 1.15 to 2.3×10^9 units/50 OD/liter. This is significantly higher (1.5×10^4 to 2.9×10^5 times) than the 7.6 to 8.0×10^3 units/50 OD/liter culture of the IFN-α obtained by Miyake *et al.* from the periplasmic space (osmotic shock/cold water wash).

6. The Examiner is further directed to the publication by Voss *et al.*, a copy of which is attached to said first Declaration as Exhibit C. In particular, Figure 3 on page 721 compares the processing efficiency of different leader sequences for IFN-α2c expression in *E. coli* W3110. Identification of the protein was done by Western blot. The migration of correctly processed IFN-α2c is marked with an "m". Figure 3 clearly shows that only the STII leader gives correctly processed product (lane 2) which is between 10 and 20 percent of the total interferon content (see page 722, right column, first full paragraph). Under the same conditions, the AP leader gives no correctly processed IFN-α2c at all! This finding is well in accordance with the Miyake *et al.* paper which reports a very small recovery (10^3 to 10^4 units/OD 50/liter) which was probably not detectable by Western blot.

8. Thus, we have unexpectedly achieved a level of recovery of IFN-α expressed from the claimed construct that is much more than 5 times greater than the recovery of IFN-α from the construct of Miyake *et al.*

9. The data disclosed in Table 1 of Chang *et al.* indicate that the level of expression of hGH from an AP/STII promoter/signal construct (0.5 g/50 OD/L) is five times higher than the level of expression of hGH from an AP/AP promoter/signal construct (0.1 g/50 OD/L). In contrast, we obtained 1.5×10^4 to 2.9×10^5 times the amount of IFN-α reported by Miyake *et al.* Thus, the results obtained with the construct of the present invention are truly unexpected.

10. I further declare that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or

imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

June 23rd, 1997
Date

Dr. Rudolf Hauptmann
Rudolf Hauptmann